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Long-Term Effects of Methylmercuric Chloride on Three Generations of Brook Trout (Salvelinus fontinalis) Toxicity, Accumulation, Distribution, and Elimination

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J.M. McKim, G.F. Olson, G.W. Holcombe, and E.P. Hunt

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16. ABSTRACT
During a 144-wk period three generations of brook trout (Salvelinus fontinalis) were continuously exposed to mean water concentrations of methylmercuric chloride (MMC) of 2.93, 0.93, 0.29, 0.09, 0.03, and <0.010 (control) µg Hg/liter. During the first 39 wk the highest concentration (2.93 µg Hg/liter) produced deformities and 88% mortality of first-generation adults. At an MMC concentration of 0.93 µg Hg/liter, second-generation trout developed deformities and all but one female died during the 108-wk exposure. No significant effects on survival, growth, or reproduction of second-generation trout were noted at any of the lower MMC concentrations. No toxic symptoms were observed in the third generation at the three lowest MMC concentrations. The maximum acceptable toxicant concentration (MATC) for brook trout exposed to MMC in this water with a hardness of 45 mg/liter (as $CaCO_3$) and a pH of 7.5 fell between 0.93 and 0.29 µg Hg/liter. The mean 96-h LC50 for yearling (200 g) and 20-wk-old (12 g) juvenile brook trout exposed to MMC was 75.0 µg Hg/liter, and the application factor (MATC/96-h LC50) lies between 0.004 and 0.013. The accumulation rate of mercury by eight selected tissues of first-generation trout exposed to MMC was relatively rapid at all MMC concentrations tested. The 2-wk tissue HG:water Hg concentration factors ranged from 1×10^3 to 12×10^3, depending on the tissue, whereas after 28-38 wk of exposure the maximum tissue Hg:water Hg concentration factors for both first- and second-generation trout ranged from 6.9×10^3 to 6.3×10^4.

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Long-Term Effects of Methylmercuric Chloride on Three Generations of Brook Trout (*Salvelinus fontinalis*): Toxicity, Accumulation, Distribution, and Elimination

J. M. McKim, G. F. Olson, G. W. Holcombe, and E. P. Hunt

U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, Duluth, Minn. 55804, USA

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During a 144-wk period three generations of brook trout (*Salvelinus fontinalis*) were continuously exposed to mean water concentrations of methylmercuric chloride (MMC) of 2.93, 0.93, 0.29, 0.09, 0.03, and <0.010 (control) μg Hg/liter. During the first 39 wk the highest concentration (2.93 μg Hg/liter) produced deformities and 88% mortality of first-generation adults. At an MMC concentration of 0.93 μg Hg/liter, second-generation trout developed deformities and all but one female died during the 108-wk exposure. No significant effects on survival, growth, or reproduction of second-generation trout were noted at any of the lower MMC concentrations. No toxic symptoms were observed in the third generation at the three lowest MMC concentrations. The maximum acceptable toxicant concentration (MATC) for brook trout exposed to MMC in this water with a hardness of 45 mg/liter (as $CaCO_3$) and a pH of 7.5 fell between 0.93 and 0.29 μg Hg/liter. The mean 96-h LC50 for yearling (200 g) and 20-wk-old (12 g) juvenile brook trout exposed to MMC was 75.0 μg Hg/liter, and the application factor (MATC/96-h LC50) lies between 0.004 and 0.013. The accumulation rate of mercury by eight selected tissues of first-generation trout exposed to MMC was relatively rapid at all MMC concentrations tested. The 2-wk tissue Hg:water Hg concentration factors ranged from 1×10^3 to 12×10^3, depending on the tissue, whereas after 28–38 wk of exposure the maximum tissue Hg:water Hg concentration factors for both first- and second-generation trout ranged from 6.9×10^3 to 6.3×10^4. The blood, spleen, and kidney accumulated mercury most rapidly and contained the highest residues in both first- and second-generation trout followed by liver, gill, brain, gonad, and muscle in order of decreasing mercury residues. There was no significant elimination of mercury from the tissue of first- or second-generation fish, yet a "steady state" (micrograms mercury per gram = constant) was reached in all tissues after 20–28 wk of continuous water exposure. Monomethylmercury made up 90–95% of the total mercury present in muscle, the only tissue analyzed for this compound. Mean muscle residues in first-generation trout, dying after 16–28 wk of exposure to 2.93 μg Hg/liter and in second-generation trout, dying after 64–100 wk of exposure to 0.93 μg Hg/liter, were 23.5 and 9.5 μg Hg/g, respectively.

McKim, J. M., G. F. Olson, G. W. Holcombe, and E. P. Hunt. 1976. Long-term effects of methylmercuric chloride on three generations of brook trout (*Salvelinus fontinalis*): toxicity, accumulation, distribution, and elimination. J. Fish. Res. Board Can. 33: 2726–2739.

Trois générations d'ombles de fontaine (*Salvelinus fontinalis*) ont été exposées de façon continue durant une période de 144 semaines à des concentrations moyennes dans l'eau de chlorure de méthyl-mercure (CMM) de 2.93, 0.93, 0.29, 0.09, 0.03 et <0.010 (témoin) μg Hg/litre. Au cours des 39 premières semaines, la concentration maximale (2.93 μg Hg/litre) produit des difformités et des mortalités de 88% des adultes de première génération. A une concentration de CMM de 0.93 μg Hg/litre, les ombles de deuxième génération souffrent de difformités et tous, sauf une femelle, meurent pendant l'exposition de 108 semaines. Nous n'avons pas observé d'effets significatifs sur la survie, la croissance ou la reproduction d'ombles de deuxième génération à aucune des concentrations plus faibles de CMM. La troisième génération ne montre aucun symptôme toxique aux trois plus basses concentrations de CMM. La concentration maximale acceptable de toxique (CMAT) pour des ombles de fontaine exposés au CMM dans cette eau d'une dureté de 45 mg/litre (comme $CaCO_3$) et un pH de 7.5 se situe entre 0.93 et 0.29 μg Hg/litre. La CL50 moyenne après 96 h pour des poissons de l'année (200 g) et des jeunes âgés de 20

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semaines (12 g) exposés au CMM est de 75.0 μg Hg/litre, et le facteur d'application (CMAT/CL50 après 96 h) se situe entre 0.004 et 0.013. Le taux d'accumulation du mercure par huit tissus choisis d'omble de fontaine de première génération exposé au CMM est relativement rapide à toutes les concentrations de CMM essayées. Les facteurs de concentration Hg du tissu : Hg de l'eau après 2 semaines varient de 1×10^3 à 12×10^3, selon le tissu, alors qu'après 28–38 semaines d'exposition, les facteurs maximums de concentration Hg du tissu : Hg de l'eau, tant pour les ombles de première de deuxième génération, varient de 6.9×10^3 à 6.3×10^4. Le sang, la rate et le rein accumulent le mercure le plus rapidement et contiennent les résidus les plus élevés, tant chez les ombles de première que de deuxième génération. Viennent ensuite le foie, les branchies, le cerveau, les gonades et le muscle, par ordre décroissant de résidus de mercure. Il n'y a pas élimination significative de mercure du tissu de poissons de première et de deuxième génération, mais on constate un « état d'équilibre » (microgrammes de mercure par gramme = constante) dans tous les tissus après 20–28 semaines d'exposition continue. Le monométhylmercure constitue jusqu'à 90–95% du mercure total présent dans le muscle, le seul tissu dans lequel nous avons dosé ce composé. Les résidus moyens dans le muscle d'ombles de première génération, morts après 16–28 semaines d'exposition à 2.93 μg Hg/litre, et dans des ombles de deuxième génération, morts après 64–100 semaines d'exposition à 0.93 μg Hg/litre, sont de 23.5 et de 9.5 μg Hg/g respectivement.

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THIS study was designed to determine the acute and chronic toxicity (involving survival, growth, and reproduction) of low concentrations of methylmercuric chloride (MMC) in water to three generations of brook trout (*Salvelinus fontinalis*) exposed over a 144-wk period, and to ascertain from the fish tissues the accumulation rate, distribution, and elimination rate of mercury. In addition, the mercury residues in trout killed by mercury during the exposure were investigated to determine tissue mercury concentrations that would be expected to cause symptoms of toxicity in brook trout.

Little information exists on concentrations of methylmercury in water that are chronically toxic to fish or that cause unacceptable residues except for the work of the following researchers: Matida et al. (1971), who exposed rainbow trout to low concentrations of MMC in both water and food and observed toxic symptoms; Hannerz (1968) who studied the accumulation and distribution of several forms of mercury, including methylmercury, in aquatic animals both in the laboratory and under field conditions; Burrows and Krenkel (1973) who observed the uptake, distribution, and half-life of methylmercury in bluegills exposed to low water concentrations for 5 days; and Olson et al. (1975), who exposed fathead minnows to low water concentrations of MMC and found no effects on survival at concentrations causing unacceptable residues. No previous studies are available on the chronic toxicity of low water concentrations of MMC to fish involving several generations and all developmental stages in the life cycle. The present study allows a comparison of first-, second-, and third-generation effects, which are of special concern when dealing with toxicants that have a high bioaccumulation potential.

Materials and Methods

PHYSICAL AND CHEMICAL

Exposure system — The exposure system consisted of a proportional diluter (Mount and Brungs 1967) that delivered 2 liters of test water per cycle to flow-splitting chambers (Benoit and Puglisi 1973). These chambers divided each of the six diluter concentrations four ways into two duplicate adult spawning tanks and two duplicate larval growth tanks. Larval growth tanks were located above and drained into the adult spawning tanks containing the same concentration of toxicant. Flow rates of test water averaged 790 ml/min for the adult spawning tanks and 120 ml/min for the larval growth tanks. The replacement time for 90% of the test water was calculated (Sprague 1969) to be approximately 4 h in both the adult spawning tanks and in each larval growth chamber. The adult spawning containers were 91.4 × 30.5 × 38.0-cm high stainless steel tanks with a 30.5-cm standpipe. Larval rearing facilities consisted of an outer glass tank, 55.0 × 27.5 × 15.0 cm, with an inside tank, 42.5 × 25.0 × 15.0 cm, divided lengthwise into two 12.5-cm wide growth chambers. Water depth in each chamber measured 12.7 cm. Gentle aeration was used in all tanks throughout the exposure. Debris was siphoned from all tanks Monday through Friday, and all tanks were scrubbed once a week. All adult spawning tanks were covered with fiberglass mesh screens and were concealed by curtains to minimize disturbance of fish. Embryos and alevins up to 4 wk old were protected from direct light by black plastic covers in nearly complete darkness (0.02 lm). Fluorescent lamps were used in combination as the main source of illumination. They were used in conjunction with incandescent bulbs wired to a light timer, which controlled the photoperiod, and an automatic dimmer, which simulated

TABLE 1. Survival, spawning, and egg production of first- and second-generation brook trout (*Salvelinus fontinalis*) exposed to various concentrations of methylmercuric chloride.

	Total mercury (μg/liter)											
	2.90[a] ± 0.122	2.97 ± 0.148	0.91 ± 0.026	0.96 ± 0.030	0.29 ± 0.020	0.29 ± 0.020	0.09 ± 0.020	0.09 ± 0.008	0.03 ± 0.002	0.03 ± 0.002	<0.01 (Control)	
Item	A[b]	B	A	B	A	B	A	B	A	B	A	B
First generation												
Adult survival during spawning (%) (Sept. 1971–Feb. 1972)	0	25	100	100	100	100	100	75[c]	100	100	100	75[c]
No. mature males	1	1	2	2	1	2	2	2	2	2	2	2
No. mature females	2	3	2	2	2	2	2	2	2	2	2	2
No. females spawning	0[d]	0	2	2	0	2	2	2	2	2	2	2
Mean spawnings per female	0	0	3	2.5	0	1.5	1	3	3	3	2	3.5
Mean viable eggs spawned per female	0	0	648	89	0	382	245	615	358	240	539	472
Second generation												
Adult survival during spawning (%) (Dec. 1973–Feb. 1974)	0[e]	0[e]	16	0[e]	0[e]	83	66	100	83	83	100	83
No. mature males	0	0	2	0	2[f]	2	2	2	2	2	2	2
No. mature females	0	0	4	0	3[f]	4	4	4	4	4	4	4
No. females spawning	0	0	0	0	3	2	2.5	2	2	2	2.5	0.5
Mean spawnings per female	0	0	0	0	2.7	4	3.7	3	4	1.5	3	1
Mean viable eggs spawned per female	0	0	0	0	296	444	0[g]	430	522	421	609	446

[a]Mean and 95% confidence interval; [b]A and B represent duplicate adult spawning tanks; [c]Disease-related deaths; [d]All spawners died before or during spawning; [e]No successful reproduction occurred in first-generation trout; therefore, there were no second-generation spawners; [f]Spawners transferred from duplicate B; [g]All fish badly diseased (no viable eggs produced).

sunrise and sunset (Drummond and Dawson 1970). Maximum light intensity measured 8.5 lm at the water surface in the adult spawning tanks.

Water characteristics — Test water throughout the exposure was obtained directly from Lake Superior. It was passed through an ultraviolet sterilizer to kill possible pathogens and was heated to the proper temperature by a hot water heat-exchange system. Throughout the juvenile–adult exposures, seasonal temperatures ranged from 9 to 15 C. Temperatures were maintained at 9 ± 1 C during all spawning, egg-incubation, and 12-wk alevin–juvenile growth studies. Total hardness, alkalinity, acidity, and pH determinations were made on all tanks, alternately, one tank a week (mean total hardness = 45 mg/liter as $CaCO_3$, range 45–46; mean acidity = 3.9 mg/liter as $CaCO_3$, range 1.9–6.1; mean alkalinity = 42 mg/liter as $CaCO_3$, range 41–44; pH = 6.9–7.5). Dissolved oxygen was measured in a different tank twice a week (mean dissolved oxygen = 7.7 mg/liter, range 4.3–12.1). Chemical measurements were made according to standard methods (APHA et al. 1971).

Toxicant solution — Nineteen liters of MMC (expressed as micrograms Hg per liter) stock solution were prepared each week and introduced from a Mariotte bottle to the diluter by a chemical-metering apparatus (McAllister et al. 1972). During the 144-wk exposure, mean water concentrations of MMC were 2.93, 0.93, 0.29, 0.09, 0.03, and <0.01 (control) μg Hg/liter. Once a week a water sample (150 ml) from one duplicate tank at each mercury concentration was analyzed for total mercury (Table 1). Total mercury in the test water was analyzed by flameless atomic absorption (Kopp et al. 1972; Olson et al. 1975). Accuracy for total mercury in water was checked by the method of known additions by adding 0.10 and 1.0 μg Hg/liter as MMC to control water and determining the percentage recovery. Water samples for recovery estimates and reagent blanks were analyzed each time samples from the test system were analyzed, and the resulting mean recovery and 95% confidence interval for the entire exposure period was 101 ± 0.18%. Precision was determined by addition of MMC at 0.10 and 1.0 μg Hg/liter to distilled water and analyzed in triplicate. This was repeated 10 times, and the results were within 2% of each other. Additional chemical tests, carried out according to the methods of Olson et al. (1975), showed that 90–92% of the mercury in the test tanks was organic. Thus little, if any, demethylation occurred in this system.

Biological

This study involved 1) exposure of yearling brook trout through spawning (May 1971–February 1972); 2) exposure of the offspring from the initial parental group through spawning (November 1971–February 1974); and 3) exposure of offspring from the second parental group to the early juvenile stage (November 1973–May 1974) (Fig. 1). Biological methods for these three generations will be given separately.

First generation — Yearlings through adult spawning — Yearling brook trout (50–70 g) were obtained from the Cedar Island Hatchery in Brule, Wisconsin. The fish were acclimated to laboratory conditions for 4 wk before starting the exposure. The experiment began in May 1971 (Fig. 1), when 32 fish were placed by stratified random assignment into each of the 12 duplicate exposure tanks.

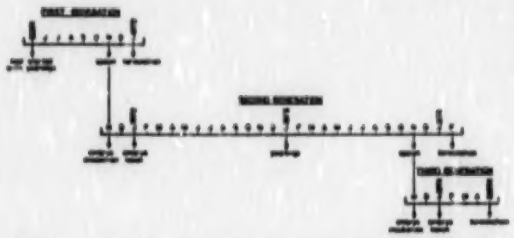

FIG. 1. Chronology of three generations of mercury-exposed brook trout (*Salvelinus fontinalis*).

Prior to spawning, fish were removed from the system after 2 and 8 wk and eight tissues (muscle, liver, kidney, spleen, brain, gonad, gill, and blood) from 4–10 trout from each mercury concentration were taken for mercury-residue determinations.

As the spawning season approached in September 1971, the remaining fish in each tank were separated into groups of males and females. From these groups fish were chosen randomly to obtain a 1:1 sex ratio consisting of two males and two females per duplicate tank. All remaining fish (4–10) in each duplicate were killed for residue analyses (same eight tissues) at 20 wk and one spawning substrate was placed in each tank. Spawning commenced in mid-November and continued into mid-December. Experimental procedures used during spawning and parental termination were the same as those described by McKim and Benoit (1971). After spawning ceased (39 wk) all remaining fish were killed for residue analysis (same eight tissues) except for two fish at each MMC concentration. These were then removed from the exposure tanks and placed in control water for 16 wk. At the end of the 16-wk period the same eight tissues, plus the remainder of the carcass, were analyzed for mercury. This allowed an estimate of the rate of loss of mercury from the tissues. Tissue samples (except for blood) were also taken from the fish that died during the 39-wk test.

All tissues were individually wrapped in cellophane, placed in sealed plastic bags, and stored at −20 C before analysis. All mercury tissues residues were determined by flameless atomic absorption (Olson et al. 1975). The reported values are based on wet weight. Accuracy for total mercury in fish tissue was checked by addition of MMC to a duplicate sample from the same homogenate. These recovery samples were analyzed with each group of tissues; usually 0.05 or 0.1 μg of MMC was added, and an overall mean recovery of 97.7% was obtained.

Second generation — Embryo through adult spawning — Exposure of the second generation of brook trout to MMC through one complete life cycle began with the spawning of the first-generation fish (Fig. 1). Biological methods used during the second-generation incubation of embryos and survival and growth studies of alevins and juveniles were the same as those described by McKim and Benoit (1974). Two hundred embryos were also transferred (as above) from the control tanks to each methylmercury concentration and fifty embryos from the two highest methylmercury concentrations to the control tank. Exposure of these transferred embryos ended 4 wk after hatching. Weights were determined on all alevins at hatch and on a subsample of 40 juvenile fish per concentration 16 wk after hatch. Second-generation juveniles were transferred from the larval growth chambers to the adult spawning tanks 12 wk after hatching.

During September 1973 second-generation fish were randomly thinned to a sex ratio of two males to four females in all tanks. Two artificial laboratory spawning substrates (Benoit 1974) were then placed in all tanks, and spawning commenced in mid-November 1973. Experimental methods used during spawning and parental termination were the same as those described for first-generation fish.

Embryos, alevins, and juveniles from all mercury concentrations and controls were sampled and preserved both by freezing and in 10% buffered formalin for later whole-body mercury-residue analysis. No detectable differences in mercury residues were measured in any tissues preserved by freezing or 10% formalin. Samples were taken at spawning, at hatch, and at 8, 20, and 28 wk after spawning. Two to six second-generation fish from each duplicate tank were also randomly selected for residue analyses at ages 40, 52, 76, 88, and 108 wk. Blood was drawn, and gill, liver, kidney, gonad, spleen, muscle, and brain tissues were dissected from these fish. After 108 wk two fish remained at each mercury concentration and were transferred to control water to determine a 12-wk dissipation rate. All blood, tissue, and remaining carcass samples from second-generation fish, including the tissues used to determine a dissipation rate, were analyzed for total mercury. The same procedures described for first-generation fish were used. In addition to the total mercury measurements made on second-generation trout muscle, the amount of methylmercury present was determined on 12 separate samples by methods described by Longbottom et al. (1973) and Talmi and Crossman (1973).

Third generation — Embryo through 12-wk-old alevins — The third-generation mercury exposure began with the spawning of the second-generation fish (Fig. 1). This generation was exposed throughout the embryo stage and for 12 wk following hatch. Biological methods were the same as those given for the second-generation alevins, except that weights of all surviving juvenile fish 12 wk after hatch were determined.

Trout diets and treatment of diseases and ectoparasites — All fish were fed measured amounts of a commercial trout food (Oregon Moist, Glencoe Mills, Silver Cup, and Ewos)[1] daily. Food rations were calculated bimonthly by multiplying the total body weight of each individual group of exposed

[1]Selection of these commercial diets was based on pesticide content and availability.

fish by a percentage of body weight ranging from 0.7 to 3.4% depending on the average weight of the fish and water temperature.

Diseases and ectoparasites were treated on several occasions throughout the exposure with neomycin, tetracycline, and formalin as recommended by The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Secondary fungal growth (*Saprolegnia*) was controlled by swabbing the infected area with a 1% solution of malachite green.

Acute toxicity tests — Two 96-h acute toxicity tests were conducted at 12 ± 1 C with yearlings (102 g) from the same group of trout used to begin the chronic test and with 5-mo-old (12-g) juvenile brook trout from another group. These continuous-flow tests with 10 fish per duplicate were conducted according to methods described by The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975).

Data handling and statistical analyses — The estimated total weight of young produced per female is the product of eggs per female, hatchability, alevin–juvenile survival, and juvenile weight. The ratio of the mean estimated total weight of young per female between fish exposed to methylmercuric chloride and controls allows a comparison of second- and third-generation results and provides one number that represents all of the observations recorded in a brook trout chronic toxicity study in relation to the controls. Spawning data, half-life data, adult and juvenile weights, and the estimated total weight of young per female were subjected to an analysis of variance with an F test of significance ($P = 0.05$) (Steel and Torrie 1960).

Results

Toxicity

First generation — During the 39-wk first-generation brook trout study, 88% of the trout exposed to MMC at a concentration of 2.93 μg Hg/liter (mean of duplicate A and B) died before or during the spawning period, and no spawning took place (Table 1). Development and behavior of first-generation trout exposed to MMC was also affected at the high (2.93 μg Hg/liter) concentration, where loss of appetite, muscle spasms, and trunk deformities appeared after 20 wk of exposure.

Spawning behavior, as evidenced by the number of spawnings produced per female, survival and growth were not affected at the lower MMC concentrations of 0.03 to 0.93 μg Hg/liter (means of duplicate A and B) (Table 1). The means and 95% confidence intervals of the lengths and weights of all test fish at spawning were 25.0 ± 1.9 cm and 187.6 ± 39.8 g, respectively. The number of viable eggs produced per female was not significantly reduced ($P = 0.05$) at any of the MMC concentrations where spawning occurred. At 0.93 μg Hg/liter (mean of duplicate A and B) the number of viable eggs produced was markedly reduced in duplicate B (Table 1), but duplicate A produced more viable eggs per female than any of the lower MMC concentrations.

Second generation — No adverse effects were seen on hatchability or on growth or survival of second-generation trout except for decreases ($P = 0.05$) in hatchability and juvenile weight at 0.93 μg Hg/liter (Table 2). When we considered the product of eggs per female, hatchability, alevin–juvenile survival, and juvenile weight as a group, and the products (estimated total weight of young per female) of each duplicate were compared, no significant differences could be shown ($P = 0.05$) at any of the four lower MMC concentrations (0.03–0.93 μg Hg/liter). However, the low ratio of the mean estimated total weight of young per female indicated a possible effect at 0.93 μg Hg/liter (Table 2).

No effects were noted on second-generation embryos transferred from the control tank at spawning to all of the MMC exposure tanks for up to 4 wk after hatching, except at the highest MMC concentration (2.93 μg Hg/liter), which resulted in no development beyond the neural keel and death of all the embryos.

Results for survival and reproduction of second-generation trout exposed to MMC throughout one complete life cycle are also presented in Table 1. No second-generation fish were produced in the 0.96 μg Hg/liter tank of the duplicate 0.93 μg Hg/liter system, so that no second-generation fish were available for spawning (Table 1). Those second-generation fish surviving at 0.91 μg Hg/liter in the other tank of the 0.93 μg Hg/liter duplicate system were all deformed in the brancheostegal–opercular region, and all but one female died during spawning. The behavior of these trout was abnormal before death; body movements were sluggish and respiratory movements were rapid. These effects during spawning at 0.93 μg Hg/liter support the earlier observations on the low ratio of the mean estimated total weight of young produced per female. The three lower MMC concentrations did not significantly affect the number of viable eggs spawned per female ($P = 0.05$), or the survival or growth of second-generation trout (Table 1). The means and 95% confidence intervals of the lengths and weights of all test fish at spawning were 28.3 ± 1.9 cm and 304 ± 50.5 g, respectively.

TABLE 2. Estimated total weight of second-generation (16-wk-old) and third-generation (12-wk-old) brook trout produced per female at various methylmercuric chloride concentrations.

Mean total mercury concn (μg/liter)	Duplicates	Estimated[a] eggs/female	× Hatchability (%)	× Alevin–juvenile survival (%)[b]	× Individual juvenile weight (g)	= Estimated total wt of young/female (g)	Ratio of mean estimated total wt of young/female (exposed : control)
			Second generation				
2.93	A	(No spawning)	—	—	—	—	—
	B						
0.93	A	583	84 (6)[c]	90	2.20[d]−0.15[e] (20)[f]	969.7	0.32
	B	80	0 (3)	0	0	0	
0.29	A	0[g]	—	—	—	—	0.77
	B	344	98 (3)	98	3.38 ± 0.08 (20)	1182.8	
0.09	A	221	100 (2)	98	2.79 ± 0.21 (20)	604.3	0.69
	B	554	99 (6)	96	2.85 ± 0.19 (20)	1500.6	
0.03	A	322	80 (5)	93	3.33 ± 0.13 (20)	797.8	0.45
	B	216	94 (2)	94	2.99 ± 0.16 (20)	563.0	
<0.01 (Control)	A	485	97 (4)	98	3.31 ± 0.57 (20)	1526.1	1.00
	B	425	97 (5)	94	3.96 ± 0.24 (20)	1534.6	
			Third generation				
2.93	A	(No spawning)	—	—	—	—	—
	B						
0.93	A	(No spawning)	—	—	—	—	—
	B						
0.29	A	158	61 (4)	80	0.94 ± 0.10 (35)	72.48	0.58
	B	236	94 (2)	98	0.96 ± 0.10 (41)	208.70	
0.09	A	—[h]	—	—	—	—	0.60
	B	229	88 (3)	90	0.81 ± 0.13 (39)	146.91	
0.03	A	278	56 (4)	85	1.18 ± 0.05 (33)	156.15	0.86
	B	224	84 (2)	100	1.40 ± 0.07 (41)	263.42	
<0.01 (Control)	A	324	71 (6)	98	0.95 ± 0.08 (44)	214.17	1.00
	B	237	87 (2)	96	1.38 ± 0.09 (40)	273.16	

[a]Number of viable eggs produced per spawning female × the probability of spawning (i.e. total spawners/total females = 0.90 for second generation and 0.5323 for third generation); [b]Fifty alevins exposed in each duplicate; [c]Number of spawnings checked for hatchability; [d]Significantly less than control ($P = 0.05$, analysis of variance with the t-test); [e]Mean and 95% confidence interval; [f]Number of fish sampled; [g]No eggs spawned in this duplicate; [h]Disease problem.

Third generation — Young third-generation trout were not significantly ($P = 0.05$) affected at the three lower MMC concentrations during the 12 wk after hatching (Table 2). The estimated total weights of 12-wk-old young per female were similar in most cases, although the ratios of the mean estimated total weights of 12-wk-old young per female were less than the control at all MMC concentrations tested.

Maximum acceptable toxicant concentration and application factor — These results establish the "no-effect level" or maximum acceptable toxicant concentration (MATC, as described by Mount and Stephan 1967) for brook trout, in water with a hardness of 45 mg/liter (as $CaCO_3$) and a pH of 7.5, between 0.93 and 0.29 μg Hg/liter. The 96-h LC50s of two continuous-flow bioassays with MMC at 12 ± 1 C for yearling (102-g) and 20-wk-old (12-g) juvenile brook trout were estimated by graphical interpolation to be 65 and 84 μg Hg/liter, respectively (mean = 75.0 μg Hg/liter). Therefore, the application factor (MATC/96-h LC50) lies between 0.013 and 0.004.

RESIDUES

First generation — Mercury accumulated rapidly in all tissues examined in first-generation brook trout (Fig. 2). After only 2 wk of exposure, tissue:water concentration factors were 1×10^3 to 12×10^3 depending on the tissue analyzed, and after 39 wk they ranged from 9×10^3 to 61×10^3.

Even the lowest MMC concentration tested (0.03 μg Hg/liter) produced a significant increase in mercury residues in all tissues analyzed (Fig. 2). The blood, spleen, and kidney accumulated the greatest quantities of mercury. The liver, gill, and brain contained the second greatest amount, and the gonads and muscle had the lowest concentration of mercury. The plasma portion of the blood contained very little mercury (3% of the total) in comparison to the high concentration in the cellular portion (97% of the total). The mercury distribution between the plasma and the red cells remained constant regardless of the mercury levels in the blood or the length of the exposure. The increase of mercury residues in gonads (male and female) after 20 wk was

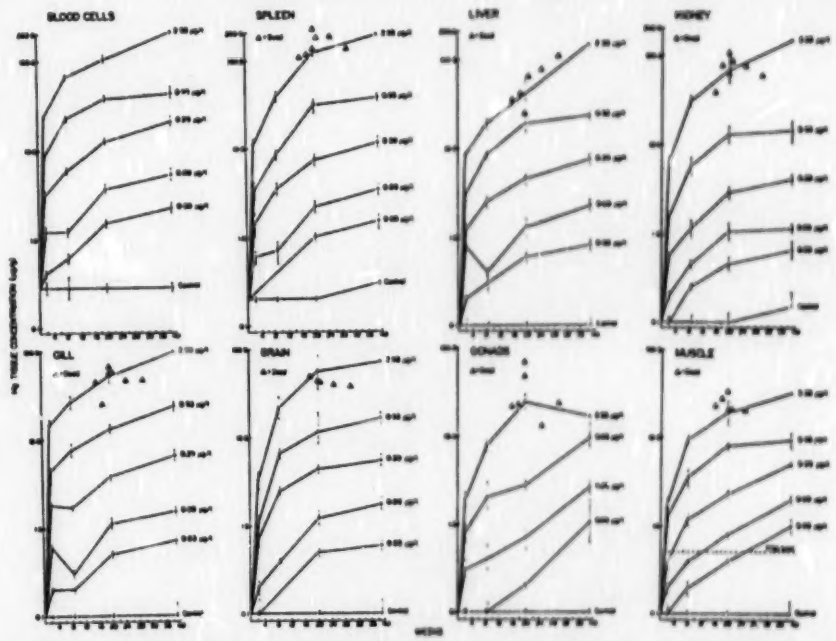

FIG. 2. Mercury concentration (micrograms Hg per gram wet weight) accumulated in selected tissues of living and dead brook trout exposed to various MMC water concentrations over a 39-wk period. Each point is the mean and standard deviation of samples from 4–10 living fish; each triangle represents a single dead fish at the highest mean MMC concentration tested (2.93 μg Hg/liter).

caused by increases in the size of these organs before spawning. The 20-wk sample was taken from "ripe" fish with well-developed large gonads, whereas the 39-wk sample was taken after spawning from fish with small gonads in various stages of regression.

Some individuals showed more resistance to toxication and as a result had higher mercury residues in certain tissues at death (Fig. 2). Brain tissue, however, varied little in the concentration of mercury present at death regardless of time of death. The mean mercury residues (micrograms Hg per gram) in each tissue at death were: spleen, 155.3; liver, 57.6; kidney, 146.6; gill, 47.7; brain, 41.6; gonad, 31.9; and muscle, 23.5.

The mercury concentration in each tissue was directly dependent on the concentration of mercury in the water (Fig. 2). Each MMC concentration in the water was approximately one-third of the next higher concentration and the concentration of mercury accumulated in tissue was also approximately one-third that found at the next higher water concentration. The concentration of mercury in the tissues tended toward a steady state with time at all MMC concentrations. However, there was no decrease in the total mercury taken up. For example, the total mercury in whole fillets of brook trout was determined and plotted in Fig. 3. This indicated that during the 39-wk exposure period there was no apparent reduction in accumulation at any water MMC concentration; in fact, the rate of uptake increased throughout the exposure.

When some of the trout were removed from the MMC exposure and placed in control water for 16 wk, the blood was the only tissue analyzed that lost a significant amount of mercury ($P = 0.05$) (Table 3). Conversely, the kidney showed a significant increase ($P = 0.05$) in total micrograms of mercury at the end of the 16-wk period. The carcass (which included all other tissues) contained 62% of the total mercury body burden; muscle, blood cells, kidney, liver, and gill (in order of decreasing body burden) made up the majority of the rest.

In contrast to the data in Table 3, the micrograms of mercury per gram of tissue dropped sharply after 16 wk in control water (Table 4). The decrease was probably caused by a growth

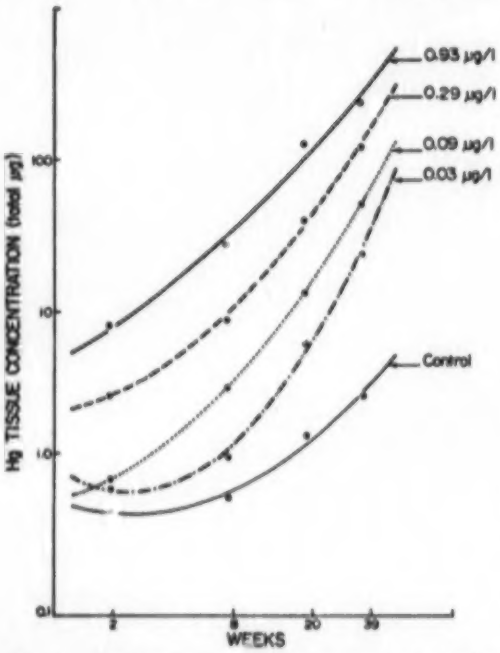

FIG. 3. Total mercury (micrograms Hg wet weight) accumulated in whole fillets of first-generation brook trout exposed to various water concentrations of MMC over a 39-wk period. Each point is the mean of four to five samples.

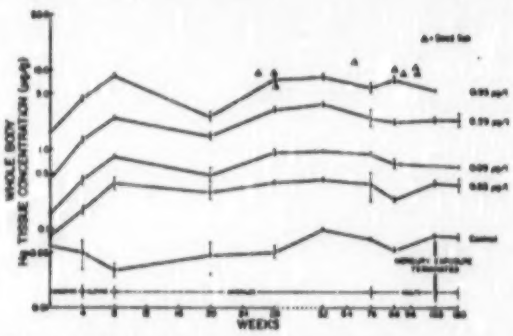

FIG. 4. Whole body mercury concentrations (micrograms Hg per gram wet weight) in living and MMC-killed second-generation brook trout exposed from embryo through adult to various water concentrations of MMC (2.93–0.03 µg Hg/liter). △, trout killed by MMC, for the curves representing residues in living trout each point (●) represents the mean and standard deviation of samples analyzed. Sample sizes analyzed were 6–50 for embryo through alevin and 1–6 for juvenile through adult.

"dilution," since the trout had increased their weight 30–45% over the 16-wk period.

Mean bioconcentration factors for a given tissue were approximately the same for all water MMC concentrations except for muscle, which showed a trend toward higher concentration factors at the lower water concentrations (Table 4).

Second and third generation — The second-generation study began with eggs from exposed first-generation parents. A considerable amount of mercury was passed from the parents to the eggs, as seen in Fig. 4 at the beginning of the embryo exposure. A separate set of residue data for third-generation trout was also analyzed, and the amount and pattern of mercury accumulation was essentially the same as in second-generation trout over the first 16 wk of exposure. Analyses of the yolk and embryos separately at hatch indicated approximately 50% of the initial mercury was contained in the yolk. The residue data for second-generation embryos at 2.93 µg Hg/liter and for third-generation embryos at 0.93 µg Hg/liter (neither shown in Fig. 4) were for embryos from stripped eggs, since no spawning occurred at these concentrations. Residues in stripped second-generation embryos at water concentrations of 2.93 µg Hg/liter and stripped third-generation embryos at 0.93 µg/liter were 12.5 ± 0.4 (mean ± SD) and 2.2 ± 0.4 µg Hg/g, respectively. All second-generation embryos were badly deformed at 2.93 µg Hg/liter and did not hatch; third-generation embryos were also deformed, and none survived more than 3 wk beyond hatching at 0.93 µg Hg/liter.

The uptake of mercury was rapid throughout the embryo–alevin stages of development at all MMC concentrations tested (Fig. 4). At the end of the alevin stage when the animals became "free-feeding," the rate of accumulation decreased between 8 and 20 wk, while accumulation rate increased between 20 and 28 wk. After 28 wk the whole-body mercury levels of second-generation brook trout had reached a steady state, and no large fluctuations were noted throughout the remainder of the exposure.

No mercury was eliminated (Fig. 4) from exposed trout 12 wk after MMC treatment ceased. The mean weight of these fish changed very little during the 12-wk period; therefore half-life values could be plotted on a microgram-per-gram basis.

During the full life-cycle second-generation exposure, toxic symptoms and deaths caused by MMC occurred only at 0.93 µg/liter and whole-body mercury residues for these dead fish were plotted in Fig. 4. The mean whole-body mercury residue for these dead fish was 9.5 µg Hg/g, which was essentially the same as residues estab-

TABLE 3. Total mercury (micrograms wet weight) in whole tissues of brook trout: A, exposed for 39 wk to various water concentrations of mercury; B, exposed for 39 wk to various water concentrations of mercury followed by 16 wk in control water. *Mean ±SE; **number of samples in parentheses; ***not measured; assumed to be approximately same as B as seen for total body burden.

Tissue	Hg water concn (μg/liter) 0.93	0.29	0.09	0.03	<0.01 (Control)	Mean % of total body burden
Brain						
A	1.4±0.20* (3)**	1.0±0.04 (3)	0.4±0.003 (3)	0.1±0.04 (3)	0.02±0.01 (3)	0.08±0.02
B	2.3±0.20 (3)	0.9±0.04 (2)	0.3±0.05 (2)	0.1±0.02 (2)	0.03±0.0 (2)	
Blood cells						
A	385.6±62.74 (3)	195.8±20.90 (3)	46.5±6.29 (3)	21.7±5.41 (3)	3.3±0.49 (3)	9.8±1.5
B	252.3±41.77 (3)	111.2±5.15 (2)	34.2±3.70 (2)	19.8±3.20 (2)	3.0±0.04 (2)	
Gill						
A	43.5±7.89 (3)	17.1±0.97 (3)	5.5±0.93 (3)	1.8±0.45 (3)	0.3±0.09 (3)	1.0±0.5
B	46.7±6.35 (3)	7.0±0.06 (2)	2.5±0.02 (2)	1.3±0.28 (2)	0.4±0.12 (2)	
Gonad						
A	4.5±1.31 (3)	3.0±0.50 (3)	0.8±0.07 (3)	0.2±0.03 (3)	0.04±0.0 (3)	0.5±0.1
B	14.1±2.84 (3)	5.5±1.25 (2)	1.5±0.12 (2)	0.8±0.20 (2)	0.1±0.005 (2)	
Kidney						
A	36.5±6.51 (3)	17.4±1.90 (3)	3.4±0.28 (3)	1.9±0.10 (3)	0.3±0.08 (3)	2.0±0.4
B	56.9±8.13 (3)	18.2±3.15 (2)	5.1±0.19 (2)	4.0±0.66 (2)	1.0±0.10 (2)	
Liver						
A	76.3±3.25 (3)	48.1±3.89 (3)	12.5±0.89 (3)	4.5±0.11 (3)	0.6±0.07 (3)	3.0±0.6
B	88.8±19.53 (3)	33.3±4.40 (2)	9.1±1.02 (2)	4.8±0.30 (2)	1.0±0.02 (2)	
Muscle (Above lateral line)						
A	422.8±19.41 (3)	209.6±19.47 (3)	97.0±10.54 (3)	42.0±4.85 (3)	5.2±0.01 (3)	21.0±2.2
B	480.2±44.68 (3)	184.8±17.70 (2)	86.0±14.8 (2)	44.0±0.50 (2)	7.4±0.40 (2)	
Spleen						
A	4.0±1.44 (3)	2.9±0.27 (3)	0.9±0.23 (3)	0.3±0.04 (3)	0.1±0.01 (3)	0.2±0.05
B	7.9±0.53 (3)	2.0±0.11 (2)	0.8±0.09 (2)	0.4±0.05 (2)	0.1±0.005 (2)	
Carcass						
A***						
B	1,593± (3)	560± (1)	209± (2)	133± (2)	25± (2)	62.0±1.5
Total body burden						
A	2,546.3±73.11	1,042.2±17.98	368.2±11.11	206.9±7.61	36.0±1.15	
B	2,487.7±237.04	922±20.43	347.9±35.49	208.7±8.85	38.1±0.90	

TABLE 4. Mercury concentration (micrograms per gram, wet weight) in the tissues of brook trout: A, exposed for 39 wk to various water concentrations of mercury; B, exposed for 39 wk to various water concentrations of mercury followed by 16 wk in control water. *Mean ±SE; ** number of samples in parentheses.

Tissue	Hg water concn (μg/liter)					Mean concn factor tissue Hg:water Hg
	0.93	0.29	0.09	0.03	<0.01 (Control)	
Brain						
A	16.9±1.52* (3)**	5.3±0.20 (3)	1.7±0.11 (3)	0.8±0.04 (3)	0.1±0.04 (3)	2×10^4
B	9.2±0.67 (3)	3.6±0.18 (2)	1.1±0.20 (2)	0.6±0.01 (2)	0.1±0.01 (2)	
Blood cells						
A	46.2±4.62 (3)	21.4±2.91 (3)	5.2±0.27 (3)	2.3±0.36 (3)	0.3±0.01 (3)	6×10^4
B	16.5±2.38 (3)	7.0±0.16 (2)	2.2±0.31 (2)	1.2±0.06 (2)	0.2±0.005 (2)	
Gill						
A	22.2±1.82 (3)	6.2±0.53 (3)	1.6±0.08 (3)	0.7±0.06 (3)	0.1±0.02 (3)	2×10^4
B	7.3±0.47 (3)	1.7±0.13 (2)	0.4±0.09 (2)	0.2±0.03 (2)	0.1±0.00 (2)	
Gonad						
A	12.3±3.73 (3)	2.9±0.53 (3)	0.9±0.41 (3)	0.2±0.01 (3)	0.05±0.00 (3)	1×10^4
B	5.0±0.78 (3)	1.4±0.29 (2)	0.4±0.13 (2)	0.2±0.03 (2)	0.04±0.01 (2)	
Kidney						
A	26.9±5.04 (3)	8.9±1.35 (3)	2.5±0.23 (3)	1.2±0.09 (3)	0.2±0.04 (3)	3×10^4
B	22.9±2.53 (3)	6.0±0.66 (2)	1.8±0.24 (2)	1.1±0.02 (2)	0.4±0.08 (2)	
Liver						
A	24.4±2.37 (3)	8.3±0.34 (3)	2.2±0.15 (3)	0.7±0.06 (3)	0.1±0.02 (3)	2×10^4
B	14.9±1.68 (3)	5.1±0.19 (2)	1.5±0.29 (2)	0.9±0.10 (2)	0.2±0.01 (2)	
Muscle						
A	10.2±1.42 (3)	4.9±0.08 (3)	1.9±0.15 (3)	1.0±0.10 (3)	0.1±0.10 (3)	2×10^4
B	5.6±0.60 (3)	2.3±0.01 (2)	0.8±0.10 (2)	0.4±0.06 (2)	0.1±0.00 (2)	
Spleen						
A	38.7±2.50 (3)	11.8±1.02 (3)	3.2±0.34 (3)	1.2±0.10 (3)	0.2±0.02 (3)	4×10^4
B	24.8±1.96 (3)	6.1±0.16 (2)	1.6±0.16 (2)	0.9±0.03 (2)	0.2±0.005 (2)	
Carcass						
B	4.4± (3)	1.6± (1)	0.59± (2)	0.4± (2)	0.1± (2)	7×10^3
Total body burden						
A	9.4±1.02	3.4±0.17	1.1±0.06	0.7±0.04	0.1±0.01	
B	4.9±0.32	1.8±0.08	0.7±0.09	0.4±0.02	0.1±0.01	2×10^4

lished for muscle alone (Table 4). Whole-body mercury residues in dead fish from unexposed parents were also analyzed. The fish from unexposed parents came from embryos transferred from the control tank at spawning and, since no natural spawning occurred in one of the duplicates at this MMC concentration, exposure of these eggs was not terminated at 4 wk as were all other transferred eggs. Fish from previously exposed parents began to die after 24 wk of continuous exposure and continued to die through the spawning period; all fish from unexposed parents died

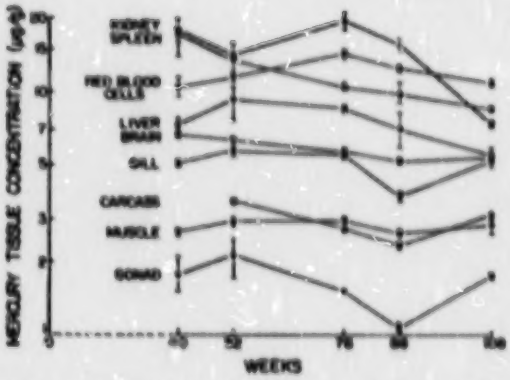

FIG. 5. Distribution of mercury (micrograms Hg per gram wet weight) in the tissues of second-generation brook trout exposed to an MMC water concentration of 0.29 μg Hg/liter. Each point represents the mean and standard deviation of five to six samples.

in 24–28 wk. The concentration of mercury in both living and dead trout from unexposed parents was considerably greater than in trout from exposed parents. The majority of dead second-generation fish from exposed parents contained more mercury (9–10 μg/g) than those surviving (7–8 μg/g). Dead fish from unexposed parents also contained more mercury (15–20 μg/g) than those surviving (11–12 μg/g).

The differences between the survival and residue data on the offspring from exposed and unexposed parents indicate possible acclimation effects.

The distribution of mercury in second-generation brook trout tissues is presented in Fig. 5 for a single MMC water concentration of 0.29 μg Hg/liter, since the tissue distribution of mercury was the same regardless of the water concentrations tested. The general distribution of mercury in these second-generation fish was the same as that described in more detail for first-generation fish (Fig. 2), although the actual concentrations of mercury were less for blood, muscle, and gill, higher for the kidney, and the same for liver, spleen, brain, and gonad. The maximum bioaccumulation found (tissue:water) in second-generation trout during the 108-wk exposure ranged from 6.9×10^3 to 6.3×10^4, depending on the tissue.

Methylmercury vs. total mercury — The amount of mercury present as methylmercury in the muscle tissue of second-generation brook trout after 88 wk of continuous exposure to various water concentrations of MMC was 99.7 ± 6.0% (mean and standard error) when computed by the method of Longbottom et al. (1973) and 90.0 ± 6.0% according to the method of Taimi and Crossman (1973). The percentage of methylmercury in the tissue was always the same regardless of the total amount of mercury present in the tissue.

Discussion

Following the exposure of three generations of brook trout to MMC, no toxic symptoms were apparent at 0.29 μg Hg/liter and below, but definite effects on survival, growth, and reproduction were observed at 0.93 μg Hg/liter and above. Therefore the maximum acceptable toxicant concentration (MATC) of MMC for brook trout is between 0.29 to 0.93 μg Hg/liter, with a calculated application factor (MATC/96-hr LC50) of 0.004 to 0.013. These data agree with those of Matida et al. (1971), who reported deaths and behavioral aberrations in rainbow trout after 10 wk of exposure at 3.7 μg Hg/liter and adverse effects on growth at low concentrations after longer exposures. The most sensitive toxic responses in this study were first indicated during the second-generation 16-wk embryo-alevin–juvenile exposure (Table 2) and were further validated during the remainder of the second- and the third-generation portions of the study. This finding is in agreement with earlier work by McKim and Benoit (1974), in which they showed that an exposure of brook trout yearlings through spawning to 12-wk-old second-generation juveniles was sufficient to predict the effects of low concentrations of copper.

The present study also indicated that brook trout in the early life history stages are more sensitive to MMC than are the older juvenile–adult trout. This early sensitivity has also been shown for other metals and other species of fish (Skidmore 1965, 1966; Hazel and Meith 1970; McKim and Benoit 1971, 1974; Pickering and Gast 1972; Eaton 1974; Pickering 1974).

The rate of mercury uptake from water by brook trout was quite rapid in all tissue studied, and the amount of mercury accumulated was directly proportional to the water MMC concentration. Concentration factors (tissue Hg: water Hg) depended on the tissue in question and ranged from 6.9×10^3 to 6.3×10^4 for both first- and second-generation trout. Second-generation trout exposed to the lowest MMC water concentration for the longest period (36–108 wk) had a muscle (and whole-body) mercury concentration factor of 1.2×10^4. The mercury concentrations in samples of brook trout

muscle were essentially the same as whole-body samples, which is understandable, since over half of a fish is muscle tissue. Huckabee et al. (1974) also found this to be true for several fish species sampled from the Great Smoky Mountains. Other researchers have found similar concentration factors for MMC. Reinert et al. (1974) reported a whole-body concentration factor of 8.0×10^3 for rainbow trout after 12 wk of continuous exposure to MMC in a flowing system and described changes in rates of uptake at different temperatures. Hannerz (1968) recorded a maximum concentration factor in northern pike of 1.5×10^3 after 4 wk of exposure, although his exposure consisted of only a single dose of MMC to a natural pond situation.

The steady-state situation seen for residues in both first- and second-generation fish was not caused by a balance between uptake and elimination, since little or no mercury was excreted by the trout (as indicated by the half-life studies in this report), and all fish continued to increase their total mercury content throughout the study. The amount of mercury being continually taken up was balanced against the continual weight gain of the trout. The steady state situation of both first- and second-generation trout seemed to be controlled by this relationship of total micrograms of mercury and total weight.

A considerable amount of mercury was passed on to the embryos from their parents. However, no natural spawning occurred in adults at water concentrations which produced embryo residues that caused deformities, low hatchability, and death. The mercury concentrations measured in alevins at swim-up were as high as any mercury concentrations found throughout the second- and third-generation exposures. These young trout were exposed to mercury transferred to their yolk from their parents and to mercury in the water, both of which contributed to the high concentrations present at this particular stage. At the end of the alevin stage, when they became "free feeding," the rate of mercury accumulation showed a marked downward trend. This was probably caused by the tremendous increase in growth (17 times) between 8 and 20 wk, which exceeded the accumulation rate of the mercury and "diluted" the mercury in the tissues. The upward trend in the mercury accumulation rate between 20 and 28 wk was probably due to a much decreased growth (2.5 times), allowing the accumulation rate of mercury to exceed the growth rate.

The distribution of mercury in the tissues of first-generation brook trout chronically exposed to low concentrations of MMC in this study agreed closely with the tissue distribution of the intravenously injected dose of methylmercuric nitrate given by Backstrom (1969) and with the stomach tube doses of MMC given by Giblin and Massaro (1973) and Miettinen et al. (1970). The route of methylmercury uptake, therefore, seemed to have little effect on the general distribution throughout the fish except for the much lower levels of mercury found in the gills of fish exposed via stomach tube (Miettinen et al. 1970). The distribution of mercury in second-generation brook trout was identical to that in first-generation trout except for an increase in the kidney, which exceeded the amount measured in all the other tissues. Takeda and Ukita (1970) found that the amount of inorganic mercury in the kidney tissue of rats increased with time after the administration of organic mercury. This may explain the high mercury content of the kidney in the second-generation trout, since they were exposed for a much longer period of time allowing for a greater period of demethylation and subsequent accumulation of inorganic mercury in the kidney tissue. The affinity of the kidney for inorganic mercury was further supported by the data of Weisbart (1973) and Backstrom (1969), who exposed several species of fish to inorganic mercury and found that fish kidney had higher concentrations of mercury than any other tissue. We also noted during the mercury-loss studies in first-generation brook trout that the blood was the only tissue analyzed that lost a significant amount of mercury, whereas the kidney was the only tissue to increase its mercury content significantly ($P = 0.05$). This change in mercury content could come about through a metabolic breakdown of the methylmercury in the blood to form inorganic mercury that subsequently moved to the kidney, as was described for mammals by Norseth and Clarkson (1971); or it might simply be due to the redistribution of mercury from the blood to other tissue. A loss from the blood with a redistribution to other tissue after a single intra-gastric dose of MMC to rainbow trout was described by Giblin and Massaro (1973). Burrows and Krenkel (1973) also presented data that indicated possible demethylation of methylmercury in bluegill kidney, although they did not indicate an increase in total mercury in the kidney.

Background levels of mercury in fish will, of course, vary with local conditions, but background concentrations of mercury in the muscle tissue of freshwater fish removed from any known source of mercury contamination have been shown to range between 0.018 and 0.20 μg Hg/g (Lofroth 1969; Kleinert and Degurse 1972;

Sumner et al. 1972; Huckabee et al. 1974). The mercury values determined for muscle samples from control brook trout in this study fell within the range established above for wild fish populations. The mean total mercury concentration of 23.5 μg Hg/g in the muscle (and whole body) tissue of first-generation brook trout that died after 16–28 wk of continuous exposure to the highest MMC water concentration (2.93 μg Hg/liter) was in close agreement with approximately 20 μg Hg/g measured in the tissue of distressed and dying fish taken from the surface of Minamata Bay, Japan, after a spill of MMC from a chemical plant (Kitamura 1968). Second-generation brook trout in the present study also died, but with much lower concentrations of mercury in the tissue (9.5 μg Hg/g) than the first-generation trout, although the exposure time was much longer (64–100 wk) and the water MMC concentration (0.93 μg Hg/liter) was lower. These differences stress the importance of both the tissue concentration and the exposure time in producing toxic symptoms in fish.

The studies described here on the direct toxic effects of MMC on brook trout show that if this form of mercury is present in low concentrations in water, then toxic symptoms ultimately will develop. However, the direct toxicity of MMC was only one aspect of these studies. The bioaccumulation of mercury in the tissues of these trout is perhaps the most important aspect of the study with respect to the environment. In 20–38 wk the lowest water MMC concentration tested (0.03 μg Hg/liter) produced methylmercury residues in the edible portions of all first-generation fish in excess of 0.5 μg Hg/g, the U.S. Food and Drug Administration's "action level" for human consumption (Federal Register 1974). The next higher water concentration (0.09 μg Hg/liter) also produced residues that exceeded 0.5 μg Hg/g in second-generation trout, but the lowest MMC water concentration causing toxic effects on the fish was 0.93 μg Hg/liter. In other words, effects on fish survival, growth, and reproduction were not observed at MMC concentrations that produced methylmercury tissue residues above the U.S. Food and Drug Administration's "action level" for human consumption. No deaths or abnormalities were observed in either first- or second-generation brook trout with whole-body residues of 2.7 μg Hg/g or less, but whole-body residues of 5–7 μg Hg/g eventually caused toxic symptoms and death. Therefore, it might be expected that environmental residues in fish between 3 and 5 μg Hg/g would be possible without direct toxic effects on the fish. Similar results were observed by Olson et al. (1975) who exposed fathead minnows to water MMC concentrations of 0.018–0.247 μg Hg/liter for 48 wk and showed the lowest concentration tested had no effect on survival yet produced whole body residues of 1.47 μg Hg/g. These relatively high tissue residues in fish brought on by exposure to low water concentrations of MMC cause even greater concern when the persistence of mercury in the tissue is considered. The present study showed essentially no loss of mercury from either first- or second-generation brook trout after 12–16 wk in control water. Several other workers have demonstrated both in the field and in the laboratory that fish show a half-life of 1–3 yr for methylmercury (Jarvenpaa et al. 1970; Lockhart et al. 1972; Burrows and Krenkel 1973; W. A. Willford personal communication). These data indicate that elimination of mercury from aquatic animals is extremely slow. Therefore, if aquatic animals are contaminated, many years would be required to completely eliminate the accumulated mercury.

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